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# CARBON DIOXIDE PRODUCTION FROM NERVE FIBRES WHEN RESTING AND WHEN STIMULATED

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# CARBON DIOXIDE PRODUCTION FROM NERVE FIBRES WHEN RESTING AND WHEN STIMULATED; A CONTRIBUTION TO THE CHEMICAL BASIS OF IRRITABILITY.[1]

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## Introduction

THERE have been two theories of the nature of conduction — one upheld among others by Hermann, that it was a propagated chemical change; the other, at present the dominant view, that it is a propagated physical change.

In 1901 Professor Mathews suggested [2] that it was in the nature of a coagulative wave propagated along the fibre; this coagulation of the nerve colloids leading either directly or indirectly to the electrical disturbance accompanying the impulse. At the time, there was no evidence of chemical change in the nerve fibre, and its indefatigability seemed to point to an absence of metabolism. Certain facts were known, however, which were difficult to reconcile with this physical theory. Darwin had observed that in Drosera,[3] conduction occurred only if the protoplasm had oxygen; and Mathews [4] observed that salts would not stimulate a nerve, or, at any rate, their power of stimulation was much reduced if the nerve remained in the body for a time after death, or if the nerve were brought into the salt solution in an atmosphere of hydrogen. This clearly indicated a dependence of the irritability on oxygen.

[1] The preliminary report of these investigations was given in part in Biochemical section of Eighth International Congress for applied chemistry, September, 1912. See original communications, Eighth International Congress of applied chemistry, xxvi, p. 163. See also this Journal 1913, xxxi, p. xxii.

[2] Mathews: Century Magazine, 1902, pp. 783–792; Science, 1902, xl, p. 492.

[3] Insectivorous Plants, p. 57.

[4] Unpublished observations.

This fact lead to a search for evidence of the chemical nature of irritability and in a number of papers [5] it was clearly pointed out that the anaesthetics were probably acting directly in a chemical manner instead of indirectly, by affecting permeability, and that probably the anaesthetics acted by uniting with the protoplasm where $O_2$ usually took hold. This view was strengthened by the temperature coefficient of conduction, which is nearly that of a chemical reaction; by the importance of $O_2$ for artificial parthenogenesis; and by many other facts some of which have recently been collected by Haberlandt, Buijtendijk and others.

Although it has been established by repeated demonstrations, that the nerve does not fatigue under ordinary conditions, as measured by the method used in muscular studies, yet Fröhlich [6] observed that the nerve undergoes certain changes by long activity. Gotch and Burch discovered [7] in 1889 that if two stimuli are successively set up within $\frac{1}{500}$ of a second, only one negative variation is produced. This critical interval, or refractory period, is found to be altered by temperature changes, by drugs, asphyxiation, and anaesthetics.[8] Thus by prolonging the refractory period by partial anaesthesia, Fröhlich easily demonstrated that with a frequency of stimulation less than this normal refractory period, stimulation of the attached muscle no longer occurred. He interprets this as a phenomenon of fatigability of the nerve. Thöner's [9] observation seems to lead to a similar interpretation, for he found recently that fatigability is less effective when the refractory period is shortened by high temperature. There seems, then, to be fatigue in the nerve, but it cannot be measured by an ordinary scale.

After the complete failure of the chemical detection of $CO_2$ and

[5] A. P. Mathews: Biological bulletin, 1904–5, viii, p. 333; this Journal, 1904, xl, p. 455; *ibid.*, 1905, xiv, p. 203; Biological Studies by the pupils of William Sedgwick, 1906, p. 81; Journal of pharmacology and experimental therapeutics, 1911, ii, p. 234.

[6] Fröhlich: Zeitschrift für allgemeine Physiologie, 1903–4, iii, p. 445. *Ibid.*, p. 75.

[7] Gotch and Burch: Journal of physiology, 1899, xxiv, p. 410.

[8] See Tait and Gunn, Quarterly journal of experimental physiology, 1908, i, p. 191; Tait, *ibid.*, 1909, ii, p. 157.

[9] Thöner: Zeitschrift für allgemeine Physiologie, 1908, viii, p. 530; *ibid.*, 1912, xiii, pp. 247, 267, 530.

acids in the excited nerve, Waller still believes that it must give off $CO_2$ when stimulated. In 1896, he showed, with an electro-physiological method, that among other reagents, $CO_2$, in minute quantities, increased the excitability of the isolated nerve of the frog, and that the normal nerve, when excited, also increased its activity.[10] From this he ingeniously formed the hypothesis that every activity in the nerve fibre must be associated with $CO_2$ production.

That there may be $CO_2$ production in the nerve, but too small to be measured by ordinary methods, is shown by the following calculations: A frog (Rana temporaria) gives off 0.355 gram of $CO_2$ per kilogram per hour at. 19 – 20° C.[11] A small piece of the nerve fibre of the same animal, say 1 cm. in length, will weigh in the neighborhood of 10 milligrams. Now, if the mass of the nerve respires at the rate of the whole animal, it would give off about 0.0000007 grams of $CO_2$ during ten minutes. This calculation at once suggested that the lack of positive evidence of metabolism in the nerve fibre was not at all conclusive that such metabolism did not occur, in view of the limitation of the methods for the estimation of $CO_2$. It was evidently necessary to devise methods for the detection of very minute quantities of $CO_2$. Thus at Professor Mathews' suggestion a new method for $CO_2$ analysis was first devised, and then, under his direction, I have undertaken to go back once more to the question of $CO_2$ production in the nerve fibre during the passage of a nerve impulse.

To study the nature of metabolism involved in a tissue, one should at least determine the oxygen consumption and the carbon dioxide production. Inasmuch, as the present problem, however, is concerned only with direct evidence for the existence of metabolism in the nerve fibre, I have attempted to measure $CO_2$ production only, for it is true that the lack of oxygen consumption may not necessarily indicate the absence of chemical changes, while the production of $CO_2$ will surely prove the presence of metabolism. Furthermore, as $CO_2$ production is the only sure universal expression of the respiratory activity in anaerobic and aerobic plant and animal tissue in normal condition, the inquiry of $CO_2$ production in an excited nerve will not only concern the problem of the nature of the nerve impulse

[10] WALLER: Croonian lecture, Philosophical transactions, London, 1896.

[11] Taken from Pott's figures. See figures in Table ix, p. 129.

itself, but may, also, aid in forming a fundamental conception of the tissue respiratory mechanism. In this way, if the protoplasmic irritability has a direct connection with the cellular respiration, then our idea of the general nature of the pharmodynamics of many reagents on a living tissue may be essentially modified.

## Methods and Materials

Two new apparati were constructed which will detect $CO_2$ in as small quantities as one ten-millionth of a gram and estimate it with quantitative accuracy. The detailed method has been described in a separate article.[12]

Preliminary experiments with these new apparati showed that the sciatic nerves of dogs gave too large quantities of $CO_2$ for my method so that I was compelled to use a smaller nerve of a cold-blooded animal for quantitative estimation. For exact measurements of $CO_2$ production, I have used only two kinds of nerve, although I have used a large variety of nerves in qualitative experiments. For a non-medullated nerve fibre, Prof. G. H. Parker[13] was so kind as to suggest to me that I use the nerve trunk of the claws of the spider crab (Labinia Caniliculata) which is a bundle of mixed sensory and motor fibres. The frog, whose sciatic was used as a representative for medullated nerve, was exclusively Rana pipiens, obtained from Indiana.

As my apparati in the present form cannot be used for a muscle nerve preparation nor for the normal nerve in situ, the use of an isolated nerve could not be avoided. Experimental factors thus introduced should be carefully considered before we interpret the observation as a normal metabolism. This serious objection, however, can be overlooked, as far as our fundamental question of different metabolic activities before and after a stimulation is concerned, for Waller[14] has demonstrated that the presence of excitability in an isolated nerve persists as long as nineteen hours provided that the electrical changes correctly represent the state of excitability. Although

[12] See pp. 137–145.

[13] For this and other suggestions, I am under great obligation to Dr. Parker.

[14] Waller: 1896, Brain, xix, p. 53.

Herzen claims that under certain conditions of local narcosis the nerve fibre may give an action current without any muscular contraction (Wedenshi and Boruttau both deny this), and Ellinson [15] recently demonstrated by the use of cinchonamine hydrochloride the absence of negative variations without abolishing the excitability of the nerve, yet evidences are now abundant to indicate that the action current is a normal physiological phenomenon in uninjured tissue expressing the simultaneous activity resulting in a corresponding change in the peripheral organ.[16] These facts, therefore, must be taken as showing that as long as a negative variation remains, the nerve is probably excitable; and that the phenomena observed in the isolated nerve could be regarded as identical with that of a normal nerve as far as the passage of a nerve impulse in an isolated nerve fibre is concerned.

## $CO_2$ Production from Resting Nerve

In this study of the metabolism of the resting nerve, particular care was taken to select those fibres which were free from nerve cells. The work of several investigators [17] seems to indicate that tissue oxidation is primarily concerned with the cell nucleus. Inasmuch as the respiration in the central nervous system is certain [18] and the blood supply to fibres is seemingly scanty, the notion persists among certain biologists that a nerve fibre should not respire since it has no nucleus. In order to test the correctness of such an idea, I have studied quantitatively the output of $CO_2$ from various lengths of nerve which are known to be free from nerve cells.[19] Here is the result:

[15] Ellinson: Journal of physiology, 1911, xlii, p. i.

[16] For further details, see: Gotch and Horsley: Philosophical transactions of the Royal Society, 1891, clxxii, p. 514; Bernstein: Archiv für die gesammte Physiologie, 1898, lxxiii, p. 376; Reid and McDonald: Journal of physiology, 1898–9, xxiii, p. 100; Lewandowsky: Archiv für die gesammte Physiologie, 1898, lxxiii, p. 288; Alcock and Seemann, *ibid.*, 1905, cviii, p. 426.

[17] See Spitzer: Archiv für die gesammte Physiologie, 1897, lxvii, p. 615; M. Nussbaun: Archiv für mikroskopische Anatomie, 1886, xxvi, p. 485; R. S. Lillie: This Journal, 1902, vii, p. 412.

[18] L. Hill: Quoted from Hulliburton's Chemistry of nerve and muscle, p. 79.

[19] In this connection, I wish to express my indebtedness to Prof. H. H. Donaldson for his kind advice.

**Non-Medullated Nerve Fibre.** — (The nerve of the spider crab, and apparatus 2 for the qualitative, and apparatus 1, for the quantitative, estimations were used.) When I place the nerve of a spider crab in the right chamber and no nerve in the left, and watch for the deposit of barium carbonate, the drop on the right will soon be coated with the white precipitate, but no precipitate whatever is visible with a lens in the left. $CO_2$ is thus shown to be produced by this resting nerve. Now, by interchanging the nerve from the right to the left, no nerve being in the right, we can convince ourselves of the correctness of this conclusion, by eliminating any technical error which might produce the different results in different chambers. The rate at which the precipitate appears and the quantity of the precipitate, depends on the size of the nerve. In fact, $CO_2$ production from the resting nerve of the spider crab is found to be proportional to its weight, other things being equal, and is constant: For 10 milligrams per ten minutes it gives $6.7 \times 10^{-7}$ grams at 15 – 16°c.

The quantitative determination of this amount is made in the following manner:

The claws of the crab are carefully removed, and, by gently cracking them, the long fibre of the nerve trunk is easily isolated. After removing the last drops of the water by a filter paper, the nerve, with the aid of glass chop sticks, is carefully placed on the glass plate,[20] and quickly weighed. The glass plate with the nerve is now hung on the platinum hooks in the respiratory chamber A, and then the chamber sealed with mercury. The analytic chamber is now filled with mercury in the manner described elsewhere,[21] and then the apparatus is washed by $CO_2$ free air as usual. The time when the barium hydroxide is introduced to the cup in chamber B is recorded, and the stop-cock between the two chambers is closed. When at the end of ten minutes the drop at cut F is perfectly clear, having not a single granule of the precipitate visible to a lens, thus insuring that the air is absolutely free from $CO_2$ then a known portion of the gas from the respiratory chamber is introduced into the chamber below in which the clear drop of barium hydroxide has been exposed, and it is determined whether or not the amount of the gas taken contains

[20] The weight of this plate is known so that the weight of the nerve can be determined very quickly. See p. 120.

[21] See pp. 139.

enough $CO_2$ to give the precipitate in ten minutes. If it does, a fresh nerve is prepared and a less volume of the gas is withdrawn; if it does not, a larger volume should be taken till the precipitate appears within ten minutes. (See footnote, page 140.)

In this way, by repeated experiments with several fresh nerves, a minimum volume of the gas for a known weight of the nerve which gives a precipitate is determined. This minimum volume should contain exactly a definite quantity of $CO_2$ — namely $1.0 \times 10^{-7}$ gram.[22]

In this way, since we know the original volume of the respiratory chamber from which this minimum volume is withdrawn, and since we know the quantity of $CO_2$ contained in this volume, it is easily calculated, how much $CO_2$ is produced by the nerve during the known period. It should be understood that in determining the minimum volume of gas taken from the respiratory chamber, a series of experiments were conducted in order to calculate both the minimum volume which just gives the precipitate and the maximum volume which does not give the the precipitate for a known weight of the nerve for a known period of respiration In the tables following, columns 8 and 9 refer to these volumes calculated from experiments.

Table I, gives the result for a non-medullated nerve.

**Medullated Nerve Fibre.** — For the quantitative estimation of $CO_2$ production from the medullated nerve I have taken a frog's sciatic, using apparatus 2. The results given in Table II, obtained by similiar methods, show that each ten milligrams of the frog's sciatic nerve gives off $5.5 \times 10^{-7}$ grams for the first ten minutes.

A large quantity of nerves were tested and it was determined whether or not all resting nerves give off $CO_2$. As a result, I found no exception in any of them. The following varieties of nerves were examined:

1. MOTOR NERVE: Occulo-motor nerve of the skate. (*Raia Ocallata.*)
2. SENSORY NERVE: Olfactory nerve of the same. (*Raia Ocallata.*)
3. MEDULLATED NERVE: Sciatic nerve of the dog, frog, turtle, mouse; optic nerve of the skate. (*Both Raia Ocallata* and *Raia Erinecia.*)
4. NON-MEDULLATED NERVES: Nerves of the spider crab; olfactory nerve of the skate. (*Raia Ocallata.*)
5. NERVE OF INVERTEBRATE: Spider crab's nerves.

[22] See p. 140.

TABLE I

$CO_2$ Production from Resting Nerve of Spider Crab, Labinia Caniliculata (Non-Medullated)

| Column 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| Date | Temperature of room | Weight of nerve in milligrams | Stimulation | Duration of respiration in minutes | Amount of gas taken from respiratory chamber | Precipitation of $BaCO_3$ after ten minutes | No. of c.c. of gas which gives precipitate, calculated for 10 mg. nerve, ten minutes duration [1] | No. of c.c. of gas which *does not* give ppt. calculated for 10 mg. ten minutes [1] | Original volume of respiratory chamber |
| Oct. 13 | 15°8 | 40 | no | 30 | 2 c.c. | + | 24 c.c. | .... | 9.5 c.c. |
| " " | 18 | 20 | " | 30 | 1 c.c. | + | 6 c.c. | .... | " |
| Nov. 3 | 16.8 | 20 | " | 10 | 1 c.c. | + | 2 c.c. | .... | " |
| " " | " | 20 | " | 10 | .5 c.c. | − | .... | 1.0 c.c. | " |
| " 4 | .... | 25 | " | 10 | .5 c.c. | + | 1.25 c.c. | .... | " |
| " " | .... | same nerve | " | 10 | .5 c.c. | − | .... | 1.25 | " |
| " 5 | .... | 16 | " | 10 | 1 c.c. | + | 1.6 c.c. | .... | " |
| " 6 | 15 | 20 | " | 10 | .5 c.c. | − | .... | 1.0 | " |
| " 7 | 14.8 | 16 | " | 16 | .5 c.c. | + | .9 [2] c.c. | .... | " |
| " " | 16 | 16 | " | 10 | 1 c.c. | + | 1.6 c.c. | .... | " |
| " " | 16 | 16 | " | 10 | 1 c.c. | + | 1.6 c.c. | .... | " |
| " " | 17.5 | 15 | " | 12 | .55 c.c. | − | .... | .99 c.c. | " |
| " " | 17 | 8 | " | 10 | .5 c.c. | − | .... | .4 c.c. | " |
| " " | 17 | 12 | " | 10 | .6 c.c. | − | .... | .72 c.c. | " |
| " " | 16 | 18 | " | 10 | .6 c.c. | − | .... | 1.08 c.c. | " |
| " 8 | 14.8 | 8 | " | 10 | 1.5 c.c. | − | .... | 1.2 c.c. | " |
| " " | " | 11 | " | 10 | 1 c.c. | − | .... | 1.1 c.c. | " |
| " " | 16 | 12 | " | 10 | .7 c.c. | − | .... | .85 c.c. | " |

[1] From these experiments, it is obvious that 1.25 c.c. out of respiratory chamber is minimum volume which gives the first precipitate. Since the original volume of respiratory chamber was 9.5 c.c. and 1.25 c.c. out of it contains the definite $CO_2$ to precipitate $BaCO_3$ which corresponds to $1.0 \times 10^{-7}$ g., total $CO_2$ production from 10 mg. of this nerve for ten minutes is calculated as follows:

$$1.0 \times 10^{-7} \times \frac{9.5}{1.25} \text{ g.} = 6.7 \times 10^{-7} \text{ g. } CO_2 \text{ at } 15° - 16°$$

[2] This abnormal result is interesting, for this nerve was found hanging down from the glass plate, touching on the mercury at one end. Whether this high production of $CO_2$ was due to this or not was not determined.

TABLE II

$CO_2$ Production from Resting Sciatic Nerve of Frog, Rana Pipiens (Medullated)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| Date | Temperature of room | Weight of nerve in milligrams | Stimulation | Duration of respiration | c.c. of gas taken from respiratory chamber | ↓ of $BaCO_3$ after ten minutes | No. of c.c. which gives ↓, calculated for 10 mg. per ten minutes [1] | No. of c.c. which does not give ↓ calculated for 10 mg. ten minutes [1] | Original volume of respiratory chamber |
| March 26 | 19° | 10 | no | 10 min. | 1 c.c. | − | .... | 1 c.c. | 15 c.c. |
| " " | ..... | same nerve | " | 20 " | 2 c.c. | + | 4 | .... | " |
| " 27 | 21 | 11½ | " | 15 " | 1.1 c.c. | − | .... | 2.47 c.c. | " |
| " " | 21 | 11 | " | 10 " | 1 c.c. | − | .... | 1.1 c.c. | " |
| " " | 28 | 6 | " | 10 " | 2 c.c. | − | .... | 1.2 c.c. | " |
| " 31 | 20 | 13½ | " | 15 " | 1 c.c. | − | .... | 2.02 c.c | " |
| " " | 20 | 14 | " | 15 " | 1 c.c. | − | .... | 2.10 c.c. | " |
| April 1 | 19.5 | 9 | " | 15 " | 2 c.c. | + | 2.70 | .... | " |
| " " | 20 | 9 | " | 15 " | 2 c.c. | + | 2.70 | .... | " |
| " " | 19 | 16½ | " | 10 " | 2 c.c. | + | 3.30 | .... | " |
| " " | 22 | 14 | " | 10 " | 2 c.c. | + | 2.8 | .... | " |
| " 2 | 21 | 11½ | " | 15 " | 1.6 c.c. | + | 2.65 | .... | " |
| " " | 25 | 12 | " | 20 " | 1 c.c. | + | 2.4 [2] | .... | " |
| " " | 24 | 10½ | " | 10 " | 2.4 c.c. | − | .... | 2.5 c.c. | " |
| " " | 23 | 13 | " | 10 " | 2 c.c. | + | 2.6 | .... | " |
| " 3 | 13 | 20½ | " | 10 " | 1.2 c.c. | − | .... | 2.46 c.c. | " |
| " " | 20 | 20½ | " | 10 " | 1.2 c.c. | − | .... | 2.46 c.c. | " |
| " " | 27 | 20 | " | 10 " | 1.2 c.c. | + | 2.40 [2] | .... | " |
| " " | 29 | 26 | " | 10 " | 1 c.c. | − | .... | 2.6 c.c. | " |
| " " | 25 | 25½ | " | 10 " | 1 c.c. | − | .... | 2.55 c.c. | " |
| " " | 18 | 22 | " | 11 " | 1 c.c. | − | .... | 2.2 c.c. | " |

[1] By glancing at the columns 8 and 9 it is clear that 2.70 c.c. is the minimum volume, for 2.6 c.c. is maximum volume which does not give the precipitate. Since original volume of respiratory chamber is 15 c.c. we have

$$1.0 \times 10^{-7} \text{ g.} \times \tfrac{15}{2.7} = 5.5 \times 10^{-7} \text{ g. } CO_2 \text{ at } 19° - 20°$$

[2] Little high result in these cases is no doubt due to high temperature.

6. NERVE OF VERTEBRATE: Nerves of frog, dog, mouse, squiteague (*cynoscion Regalis*), and skate. (*Both Raia Ocallata* and *Raia Erinecia.*)
7. NERVE OF WARM-BLOODED ANIMALS: Those of dog, mouse and rabbits.
8. NERVE OF COLD-BLOODED ANIMALS: Frog, squiteague (*cynoscion Regalis*) and skate. (*Both Raia Ocallata* and *Raia Erinecia.*)

From this I have concluded that isolated nerves of all animals give off $CO_2$. It remains, now, to consider whether this $CO_2$ is the product of normal respiratory activity or due to disintegration of the dead tissue.

## IS THE $CO_2$ GIVEN OFF PRODUCED BY LIVING PROCESSES?

**Comparison of Dead and Living Nerves.** — In the first place, it was thought that if $CO_2$ was due to normal metabolism of a living nerve, its production should be diminished when the nerve was killed. The following result (Table III) is self explanatory.

TABLE III

COMPARISON BETWEEN NORMAL AND KILLED (BY STEAM) NERVES OF SPIDER CRAB

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| Date | Temperature of room | Weight of nerve in mg. | Stimulation | c.c. of gas taken from respiratory chamber | Duration of respiration: minutes | Ppt. of $Ba(CO_3)$ after ten minutes |
| Nov. 4 | 13° | 40 (killed) | no | .5 | 10 | – |
| " " | .. | 40 (killed) | st'n | .5 | 10 | – |
| " 5 | .. | 16 (normal) | no | 1. | 10 | + |
| " 6 | 15 | 16 (killed) | no | 1. | 12 | – |
| " 7 | 16 | 16 (normal) | no | 1. | 10 | + |

**Comparison of Anaesthetized and Non-Anaesthetized Nerves.** — It is naturally feared, however, that the killing experiment itself may not prove that $CO_2$ production is necessarily due to the living mechanism, for high temperature may drive off $CO_2$ produced already by the process of tissue disintegration, just as the $CO_2$ diffused out from a wet thread saturated with the gas, the rate of diffusion being a function of temperature. Thus anaesthesia was tried, although we should

expect at the outset that if ether had no direct affect on the respiratory process, as some physiologists believe, then the negative results would not at all interfere with my contention. The fact is, however, that either an isolated nerve directly treated with ether vapor or urathane, or the nerve isolated from a deeply anaesthetized frog gave a much less quantity of $CO_2$ than the normal nerve isolated from a normal frog whose heart has been cut away for a period of time equal to that of etherization. Anaesthetics, then, diminish $CO_2$ production from an isolated nerve fibre. These experiments are being continued quantitatively.

**$CO_2$ Production of Isolated Nerve at Successive Time Intervals.** — It was also thought that if $CO_2$ production was due to bacterial decomposition, although it is highly improbable for such a fresh tissue, we may expect that either killing by steam or treating with

TABLE IV

SHOWING DECREASED $CO_2$ PRODUCTION BY LONG-STANDING (FROG'S SCIATIC)

| 1 | 2 | 3 | 4 |
|---|---|---|---|
| Temperature of room | Time elapsed after isolation | Minimum c.c. necessary to give ↓ calculated for 10 mgs. 10 minutes | Total $CO_2$ produced from nerve of 10 mg. for 10 minutes |
| 24° | immediately | 2.7 c.c. | $5.5 \times 10^{-7}$g. $CO_2$ |
| 25 | 1 hour | 7.08 c.c. | $2.1 \times 10^{-7}$g. $CO_2$ |
| 24 | 2 hours | 10.8 c.c. | $1.4 \times 10^{-7}$g. $CO_2$ |
| 24 | 5.5 hours | 12.8 c.c. | $1.1 \times 10^{-7}$g. $CO_2$ |
| 23.5 | 7 hours | 15.3 c.c. | $.9 \times 10^{-7}$g. $CO_2$ |
| 23.5 | 10.5 hours | 21.0 c.c. | $.6 \times 10^{-7}$g. $CO_2$ |
| 24 | 26 hours | 9. c.c.[1] | $1.6 \times 10^{-7}$g. $CO_2$ |
| 24 | 27.4 hours | 1.8. c.c | $8.1 \times 10^{-7}$g. $CO_2$ |

[1] The gradual increase at this point should be noted (after 26 hours, it is clear that bacterial decomposition sets in).

ether would check the $CO_2$ production, and that the results observed above may not necessarily prove that $CO_2$ production from the isolated nerve fibre is due to a respiratory process. Hence a number of the nerves were isolated from several frogs of the same size and sex, and

were left in Ringer's solution, and then the rate of the gas production is determined with the different nerves removed at successive intervals of time from the Ringer's solution for twenty-five hours. The interesting results given in Table IV not only show that $CO_2$ from the fresh nerve is not due to bacterial decomposition, but it also indicates that when such abnormal decomposition sets in, the output of gas takes a sudden jump. This Table further shows that the vital process by which $CO_2$ is produced gradually slows up as the tissue approaches death, indicating that the decrease of $CO_2$ production is parallel to the decrease of irritability of the nerve.

**Increase of $CO_2$ on Stimulation.** — The most convincing evidence of all that $CO_2$ is formed by a vital process is the fact that a stimulated nerve gives off more $CO_2$ (Part II) indicating the presence of normal metabolism in the living nerve which is accelerated when the nerve is stimulated. Thus we may safely conclude here that like any other tissue or organs, the nerve, too, respires whether it has a nucleus or not, and that the rate of $CO_2$ production is proportionate to its weight, other things being equal.

## $CO_2$ Production from Stimulated Nerve

We have now come to our main inquiry, namely, is there any chemical basis for irritability? Just what relation exists between nervous activity and chemical changes is the question that a biologist should consider before he attempts to build any conception of the real dynamics of living matter. For it is the phenomena of excitability in the nerve fibre that has stood so long in the path of understanding protoplasmic irritability in general. As for the brain, it is now established that certain chemical changes are involved during stimulation and that definite chemical changes are associated with pathological cases either in its chemical composition [23] or in the formation of abnormal metabolites.[24] Aside from the confused facts concerning histological changes in the ganglion cells of fatigued animals, Hill has observed, using Ehrlich's method of methylene blue

[23] Koch and Mann: Archiv of neurology and psychiatry, 1909, iv, p. 44.

[24] Dixson: Journal of physiology, 1899–1900, xxv, p. 63; Croftan: American journal of the medical sciences, 1902, p. 150.

for the determination of the rate of oxidation, that a spot of cerebral surface, if stimulated, loses its blue color owing to the using up of the oxygen.[25] In case of the nerve fibre, however, we have already seen that no direct evidence has ever been presented to show any chemical changes connected with its activity, although there has been some indirect evidence. As considered before, the failure of the direct detection of $CO_2$ from the stimulated nerve must be due to the lack of a delicate method. Thus using the new method we have already demonstrated that a resting nerve gives off $CO_2$, and will now attempt to prove that nerves give off more $CO_2$ when stimulated.[26]

**Electrical Stimulation of non-Medullated Nerve.** — Owing to the scope of delicacy of the new method, which is sensitive to as small a quantity as $1.0 \times 10^{-7}$ gram (an amount corresponding to the $CO_2$ contained in $\frac{1}{6}$ cc. of pure air), the utmost caution must be taken to prevent any complication which may result in formation or absorption of minute quantities of $CO_2$. After I had found by experiment that there is no appreciable increase of $CO_2$ due to the direct electrical decomposition in the nerve when stimulated by a weak induction current and that several other forms of stimulation qualitatively confirmed the results obtained by the electrical stimulation, I have naturally employed the induction current as a stimulant in all my experiments on the quantitative estimation of $CO_2$ production from the stimulated nerve.[27]

As Table V shows, the stimulated non-medullated nerve fibre of the spider crab gives off $16. \times 10^{-7}$ grams of $CO_2$ for 10 milligrams of

[25] HILL: *loc. cit.*

[26] Professor Carlson has very kindly called my attention to a recent publication from the Physiologisch Laboratorium der Utrechtsche Hoogeschool, in which Buijtendijk reports that certain head nerves of fishes take up more $O_2$ when electrically stimulated. He could not, however, find any increase of $O_2$ consumption in the sciatic of the frog. Also see: Koninklijk Akademie van Wetenschappen, Amsterdam, afd, xix, pp. 615–621.

Haberlandt also recently reports (Archiv für Physiologie, 1911, p. 419) that the resting nerve takes up of $O_2$, $41.7 - 33.4$ cmm. at $19° - 24°$ per gram per hour. When this nerve is excited, intake of $O_2$ is increased. Since the respiratory quotient of the stimulated nerve is equal to that of the resting, he concludes that when the nerve is excited, it must give off more $CO_2$. He does not, however, indicate how much $CO_2$ is produced by stimulation.

[27] Use of non-polarizable electrodes was impossible for my apparatus, for the presence of foreign liquid in the chamber interferes with $CO_2$ estimation. As

nerve for ten minutes, while a fresh resting nerve gave only 6.7 by $10^{-7}$ grams for the same units. The details of the methods are as follows:

The nerve of the claw of the spider crab is isolated as before. A comparative estimation was made first. Two pieces of the nerve of equal weights and length were placed separately on the two glass plates, each nerve being laid across the electrodes of the plate, in the manner shown in Figure 1. In this way either nerve can be stimulated at will. These glass plates are hung by their wires upon the platinum wires fused into the side of the apparatus, these wires being connected in turn with the induction coil. Under this condition, when both nerves are not stimulated, the amounts of the precipitate are equal in both chambers. However, when one of the nerves is elec-

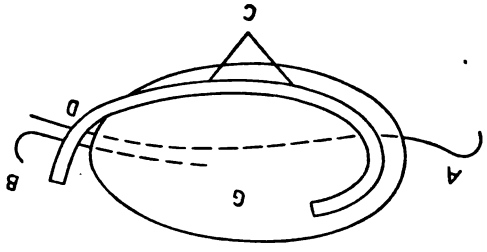


FIGURE. 1. Glass weighing plate. A. B. Platinum wire fused in the rear of the glass plate, with hooks. C. The nerve which is stimulated at D. G. The plate proper.

I have the other piece of the same glass out of which this plate is made. This piece of glass is weighed exactly equal to this weighing plate, so that any wet tissue can be weighed very quickly. In order to make results more accurate, no attempt was made to weigh closer than ½ milligram.

trically stimulated (the distance between the primary and secondary coils was always more than 10 cm. using a red dry battery, the current being barely perceptible on the tongue), not only does the precipitate appear sooner in the chamber in which the excited nerve is placed, but also the quantity of the carbonate is much greater.

To test whether the increase of $CO_2$ production from the stimulated nerve is due to the direct decomposing influence of the current, or to the increase of metabolism produced by the passage of a nerve

long as we are not concerned with the electrical changes in the nerve, the use of platinum electrodes instead, is not a great objection, provided that the current is weak enough not to decompose the tissue directly, and that the duration of stimulation is not very long.

impulse, the following experiments were performed. If we assume that the condition under which an electrical decomposition takes place is the same both in the living and the dead nerve, then if the increased $CO_2$ is due to the current itself, we should expect that when a killed nerve is stimulated by a current, it ought to increase $CO_2$ production just as much. When I placed two nerves killed by steam in each chamber, and stimulated only one of them, the stimulated nerve did not give any more $CO_2$ than the unstimulated, using the same strength of current employed in the other experiments. In the next place, it was thought that if the increase of $CO_2$ is due to direct electrical decomposition, not limited to the point of contact with the electrodes, we ought to get a proportional increase of $CO_2$ by altering the distances through which the current directly passes. The fact was, however, that we could produce an increase of $CO_2$ production by stimulating with electrodes 2 mm. apart as well as by 15 mm. apart. Increase of $CO_2$, therefore, is due to nervous excitation and not to the direct influence of the electric current itself.

With this consideration, I have proceeded to make a quantitative estimation of $CO_2$ from the stimulated nerve in the manner described before. The results are shown in Table V.

**Electrical Stimulation of Medullated Nerve.** — With apparatus 2, the output of $CO_2$ from the excited sciatic nerve of the frog has been quantitatively estimated. As shown below, 10 mgs. of the sciatic nerve gives off $14.2 \times 10^{-7}$ grams of $CO_2$ during ten minutes stimulation while the resting nerve of the same animal gave off $5.5 \times 10^{-7}$ grams for the same units.

**Mechanical Stimulation.** — We have now established the fact that when a nerve is stimulated by an electrical stimulus, it gives off more $CO_2$. In order to prove more conclusively that this $CO_2$ production is due to the passage of a nerve impulse, I have employed several other means which are known to have definite influence on excitability of the nerve. So far, the use of these methods has been confined to qualitative experiments, but the results are a sufficient confirmation of the observations made by electrical stimulation. I cite them here as a preliminary report.

Since the ordinary method for mechanical stimulation cannot be applied directly to the nerve in my apparatus in its present form, I used a different method, namely, crushing the nerve. That, when a

TABLE V

$CO_2$ Production from Stimulated Nerve of Spider Crab (Non-Medullated)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| Date | Temperature of room | Weights of nerve in milligrams | Stimulation | Duration of stimulation | c.c. taken from respiratory chamber | Ppt. of $BaCO_3$ after ten minutes | No. of c.c. of gas which gives ppt. calculated for 10 mg., ten minutes [2] | No. of c.c. of gas which does not give ↓, calculated for 20 mg., ten minutes [2] | Original volume of respiratory chamber |
| Nov. 3 | 16.8° | 20 | Stimulated | 10 min. | .5 | + | 1 c.c. | ... | 9.5 |
| " 3 | 14 | 12 | " | 10 " | .5 | + | .6 c.c. | ... | " |
| " 4 | 14 | 40 | " | 10 " | .5 | − [1] | ... | 2.0[1] | " |
| " 6 | 14.8 | 20 | " | 10 " | .5 | + | 1 c.c. | ... | " |
| " 7 | 16.8 | 8 | " | 10 " | .5 | + | .4 c.c. | ... | " |
| " " | 16.5 | 8 | " | 10 " | .5 | − | ... | .4 | " |
| " " | 17 | 16 | " | 10 " | .5 | + | .8 c.c. | ... | " |
| " " | 17.4 | 16 | " | 10 " | .5 | + | .8 c.c. | ... | " |
| " " | 17.5 | 8 | " | 10 " | .5 | − | ... | .4 | " |
| " " | 17 | 8 | " | 10 " | 1.0 | + | .8 c.c. | ... | " |
| " 8 | 15 | 11 | " | 10 " | 1.0 | + | 1.1 c.c. | ... | " |
| " " | 16 | 10 | " | 10 " | 1.0 | + | 1 c.c. | ... | " |

[1] Killed by steam.

[2] From this we see minimum precipitating volume is .6 c.c., since .4 c.c. is maximum non-precipitating volume:

$\therefore$ Total $CO_2$ output from 10 mg., ten minutes is

$1.0\times10^{-7} \times \frac{9.5}{.6}$ g. $= 16 \times 10^{-7}$ g. $CO_2$ at 14° − 16° Compare with Table I

TABLE VI

$CO_2$ PRODUCTION FROM STIMULATED SCIATIC NERVE OF FROG (MEDULLATED)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| Date | Temperature of room | Weight of nerve in milligrams | Stimulation | Duration of stimulation | c.c. taken from respiratory chamber | Precipitation of $BaCO_3$ after ten minutes | No. of c.c. of gas which gives ↓, calculated for 10 mg., ten minutes [1] | No. of c.c. of gas which does not give ↓, calculated for 10 mg., ten minutes [1] | Original volume of respiratory chamber |
| March 27 | 24° | 14 | Stimulated | 10 min. | 2 | − | .... | .8 c.c. | 15 c.c. |
| " " | 24 | 13 | " | 10 " | 1 | + | 1.3 | .... | " |
| " " | 24 | 11.5 | " | 10 " | 1 | + | 1.15 | .... | " |
| " 28 | 18 | 13 | " | 10 " | 1.8 | + | 2.34 | .... | " |
| " " | 19 | 8 | " | 20 " | 1 | + | 1.6 | .... | " |
| " " | 20 | 9.5 | " | 20 " | 1 | + | 1.9 | .... | " |
| " " | 20 | 15 | " | 10 " | 1 | + | 1.5 | .... | " |
| " 30 | 25 | 14.5 | " | 10 " | 1 | + | 1.45 | .... | " |
| " " | 24 | 9 | " | 11 " | 1 | − | .... | .99 c.c. | " |
| " " | 21 | 9 | " | 10 " | 1.5 | + | 1.35 | .... | " |
| April 3 | 18 | 22 | " | 10 " | .9 | + | 1.98 | .... | " |
| " 4 | 21 | 17 | " | 10 " | 1 | + | 1.7 | .... | " |
| " " | 22 | 10.5 | " | 10 " | 1 | + | 1.05 | .... | " |
| " " | 25 | 16.5 | " | 10 " | 1 | + | 1.65 | .... | " |
| " " | 23 | 1.6 | " | 10 " | 1 | + | 1.6 | .... | " |
| " " | 21 | 1.7 | " | 10 " | 1 | + | 1.7 | .... | " |
| " 5 | 25 | 9.7 | " | 10 " | 1 | − | .... | .97 c.c. | " |
| " " | 26 | 14 | " | 10 " | 1 | + | 1.4 | .... | " |

[1] Since minimum precipitating volume is 1.05 c.c. and maximum non-precipitating volume is .99, it is obvious that 1.05 c.c. is minimum:
$\therefore 1.0 \times 10^{-7} \times \frac{15}{10.5}$ g. $= 14.2 \times 10^{-7}$ g. $CO_2$ (20° − 22°)

protoplasm is smashed, there occurs vigorous chemical changes, is shown by several investigators. Fletcher [28] reports that injured muscle gives off more $CO_2$ than the normal.

Later he and Hopkins [29] discovered that muscle, under a similar condition, is richer in lactic acid.

Dr. Mathews has observed a similar activity in crushed eggs of Arbacia. Quite accidently, I have discovered that a fresh nerve, too, when crushed with the rough edge of a glass rod gives off more $CO_2$. This increase of gas production from the injured nerve, I take to be due to mechanical stimulation. To test this hypothesis, I rendered the nerve unexcitable by means of ether and 0.2 m. solution of KCl, which is known to abolish excitability of a nerve.[30]

Under these conditions, I observed no increase of gas production when the nerve is crushed. Therefore, the metabolism existing in the living nerve must be accelerated by this stimulation when it is injured.

This interpretation, however, is not accordant with that of Fletcher and Hopkins, on muscle. In studies of lactic acid formation in muscle, they found that lactic acid is spontaneously developed, under anaerobic condition, in excised muscle, and that fatigue due to contractions of excised muscle is accompanied by an increase of lactic acid. In an atmosphere of $O_2$, there is no survival development of lactic acid for long periods after excision. From a fatigued muscle, placed in $O_2$, there is a disappearance of lactic acid already formed. But this disappearance of lactic acid, due to oxygen, does not occur, or is masked, at supraphysiological temperature (e. g., at 30 ). Now traumatic injury to an irritable muscle too produces a rapid development of acid. Since, however, in this case the disappearance of lactic acid due to $O_2$ does not occur, they conclude that one essential condition for this effect of oxygen appears to be the maintenance of the normal architecture of the muscle. Thus they contend that the increase of the lactic acid by mechanical injury is not due to stimulation, but must be due to tissue destruction.

They, however, did not determine, as far as I know, how much the output of $CO_2$ is affected by treating the injured tissue with $O_2$.

[28] FLETCHER: Journal of physiology, 1898–9, xxiii, p. 37.
[29] FLETCHER and HOPKINS: *ibid.*, 1906–7, xxxv, pp. 261, 288.
[30] MATHEWS: This Journal, 1904, xi, p. 463.

Unless it is proven that $CO_2$ production from the injured muscle is quantitatively equivalent to lactic acid formed, their interpretation cannot be applied to the injured nerve, for in the case of the "plateau" of the survival muscle respiration, when in complete loss of irritability, the lactic acid yield remains stationary, Hill calculated that the $CO_2$ production corresponds to the amount liberated from the carbonate of the tissue by the lactic acid formed.[31]

Furthermore, if their interpretation is applied to the nerve, the fact that etherized nerves or nerves rendered unexcitable by KCl do not increase $CO_2$ output when crushed, cannot be explained. The fact that only excitable nerves when injured increase their $CO_2$ production, is a sufficient proof that some sort of stimulation is applied to the nerve when crushed, the tissue destruction, no doubt, following afterward. The increase of $CO_2$ production on crushing the living nerve and its absence on crushing the anaesthesized nerve is the point that I want to emphasize here in order to confirm my results obtained by electrical stimulation. I may add here that a perfectly parallel increase of $CO_2$ by crushing has been observed in dry seeds, including wheat, wild oats, Lincoln oats, Swedish select oats, leaves of Japanese ivy, and spinal cords of rabbit.[32]

**Chemical Stimulation.** — The study of the nature of chemical stimulation has been so thoroughly made [33] that at first it was thought that chemical reagents would be ideal as stimuli.

It was soon discovered, however, that the presence of minute quantities of a foreign liquid is such a disturbing factor that stimulation by salt solutions could not be used for quantitative experiments. With a qualitative analysis, however, I found a variety of evidences which show that the nerve stimulated chemically gives off more $CO_2$, and that the nerve rendered less excitable by reagents decreases $CO_2$ production.

When each sciatic nerve of a frog is isolated and one is left in the normal saline in one case, and in the body of the frog in the other, for the same length of time, and then transferred to the two chambers of the apparatus, if the quantities of the precipitate are compared, it is found that the nerve which has been in normal saline gives more $CO_2$.

[31] HILL: Journal of physiology, 1912, xliv, p. 481.

[32] Fuller discussion of these will appear in a subsequent paper.

[33] MATHEWS: This Journal, 1904, xl, p. 455; 1905, xiv, p. 203.

It is known that normal saline stimulates frog's sciatic nerves. The different rates at which $CO_2$ is produced from the different nerves treated by various concentrations of KCl is equally instructive. It is known that when a nerve is placed in a molecular solution of KCl, a stimulation takes place for a considerable time. Then it finally becomes unexcitable,[34] whereas, .2 m. KCl solution abolishes nervous excitability in a short time without primary stimulation. The $CO_2$ production follows exactly analogous to this. The nerve treated with the stronger solution gives more $CO_2$ than that of a weaker solution. This was true even after both nerves became unexcitable, showing that the nerve must be giving off more $CO_2$ while being stimulated by the stronger solution. Although my quantitative data are not complete at this stage, this preliminary statement is sufficient to show that the nerve chemically stimulated gives off more $CO_2$. It may be added in passing that the different solubility of $CO_2$ in the different concentrations of these salts solutions cannot explain these results solely by a physical interpretation, for there is not enough difference in the solubility of $CO_2$ in dilute equimolecular solutions of KCl, and NaCl, whose effect on $CO_2$ production is so divergent, the former salt diminishing, the latter increasing it.

**Heat Stimulation.** — It may be recalled in Table I that high temperature increases the output of $CO_2$ from the resting nerve. A respiratory process should increase proportionally to the temperature. Raising of temperature, however, not only increases the rate of respiration, but also (particularly by sudden changes of it) stimulates the nerve. A very interesting fact is observed in connection with the killing of the nerve. When the nerve is killed gradually by a slow increase of temperature, it gives off more $CO_2$ than when killed suddenly, the determination being made after both are killed. $CO_2$ production from the dead nerve under this condition must be due to the diffusion of the gas which was formed previously, just as Fletcher's dead muscle is charged with $CO_2$ gas. The different outputs of $CO_2$ between slowly killed and suddenly killed nerves cannot be accounted for unless we assume that in one case, $CO_2$ is produced more while being killed than in the other. Whether such increase of $CO_2$ production, however, was due to the acceleration of normal respiration by the slowly increasing temperature, or due to direct stimulation caused

[34] MATHEWS: *loc. cit.*

by heat, or due to both, cannot be decided here unless we consider the relation between excitation and tissue respiration.[35]

It is hoped that we may have a better understanding of this matter when we study the temperature coefficient of normal respiration of the nerve. At present, we are satisfied to state only that there is a strong evidence to support the conclusion that heat, too, increases $CO_2$ production from the nerve.

## Discussion of the Results

**Comparison of Metabolism of Non-Medullated and Medullated Nerve.** — Although it appears ridiculous to attach any significance to the marked similarity in the magnitudes of $CO_2$ production from non-medullated and medullated nerves, the temptation is irresistible to comment on the high output of $CO_2$ from the non-medullated nerve fibre. Let us study the Table following (Table VIII), in which a summarized comparison is given.

TABLE VIII

| Nerve | $CO_2$ from resting nerve | $CO_2$ from stimulated nerve | Rate of increase of $CO_2$ |
|---|---|---|---|
| Non-medullated (spider crab) | $6.7 \times 10^{-7}$ g. (15° – 16°) | $16. \times 10^{-7}$ g. (14° – 16°) | 2.4 times |
| Medullated (frog) | $5.5 \times 10^{-7}$ g. (19° – 20°) | $14.2 \times 10^{-7}$ g. (20° – 22°) | 2.6 " |

Since I have found that injury increases the $CO_2$ production from the nerve, the values I have obtained from cut, or isolated, fresh resting nerves, such as I had to use, may be somewhat greater than the output of normal uninjured nerves would be. But since Alcock[36] has shown that a non-medullated nerve gives a higher electrical response, both in the negative variation and the injury current, the $CO_2$ increase due to the cut alone will probably be greater in case of the non-medullated nerve than in that of the medullated one. That means that the value of the $CO_2$ production for the resting uninjured,

[35] See p. 134.

[36] Alcock: Proceedings of the Royal Society, 1904, lxxiii, p. 166.

non-medullated nerve should be reduced more from the figures found for the isolated nerve, than that of the medullated one. In other words, by lowering $6.7 \times 10^{-7}$ gram which is the value for resting, non-medullated, isolated nerves, the rate of increase of $CO_2$ by stimulation in the uninjured nerve would become higher than 2.4 times, and probably higher than 2.6 times, which is the rate for the medullated nerve. This greater effect in the non-medullated nerve is what we should expect if our present conception that conduction is in the axis cylinder only, is correct. Before any accurate comparison of the increase of $CO_2$ production on stimulation of non-medullated and medullated nerves can be made it will be necessary, however, to determine how much of the $CO_2$ from the resting nerve is due to injury alone. Before we consider this point seriously, also, we should determine the metabolic activities of greater numbers of nerves of different animals. Such an investigation is at present useless until we determine more quantitatively the relation between $CO_2$ production and the various strengths of stimulation and the degree of excitability. If any uniformity of $CO_2$ output in respect to anatomical variations is discovered, light may be thrown on the function of the medullary sheath and other differentiations.

However insignificant these results may be as far as the similar rates of the gas production of these two nerves is concerned, it should be strongly emphasized that technical error plays no part in these determinations. Inasmuch, as we are dealing with such an extremely small amount of the gas, it is quite natural for those who are not familiar with my apparati to suspect, by a hasty inspection of my results, that the small differences I found under different metabolic conditions may be due to mere experimental variations. For this reason, particular attention is called to a detailed description of the quantitative method I used, especially the footnote on page 144, where I have cited a series of determinations of unknown quantities of $CO_2$ in testing my apparati. I may repeat here that my experiments with the spider crab and the winter skate were done at Woods Hole[37] during the summer of 1911, while those with the frog were done in Chicago during the winter of 1912. Under these different conditions, I have not only used the different sizes of nerves, but also

[37] I take great pleasure in acknowledging my indebtedness for the kind accommodation offered me by Drs. Lillie and Drew at Woods Hole.

experimented with two different apparati, the respiratory chambers of which have had entirely different capacities.[38]

**Comparison between the Metabolism of Resting Nerves and that of Other Tissues.** — To compare the rate of metabolism of the nerve with that of other tissues is a matter of no great physiological value on account of great variations which do not affect equally the rate of $CO_2$ production. Simply to give a better picture of the scope of nervous metabolism, however, let us make the following comparison: Since there is no exact determinations made on either the other organs, or the whole animal, in the case of the spider crab, I have quoted those of the nearest crustacea of which data are available. (Table IX).

TABLE IX

| Animals | $CO_2$ per Kg. per hour | Temperature | Determined by [1] |
|---|---|---|---|
| Crustacea (whole animal) | | | Jolyet and Regnaut |
| Cray fish (*Astacus*) . . . . . | 37.7 c.c. | 12°.5 | " " " |
| Crab (*Cancer pagurus*) . . . . . | 89.9 c.c. | 16 | " " " |
| Lobster (*Homarus vulgaris*) . . . . | 54.4 c.c. | 15 | " " " |
| Nerve of spider crab (*Labinia caniliculata*) . . . . . | 212 c.c. | 15° – 16° | Tashiro |
| Frog: (*Rana esculenta*) (whole animal) . | .082 gms. | 17 | Schultz |
| (*Rana temporaria*) (whole animal) | .355 " | 19° – 20° | Pott |
| (*Rana pipiens*) (sciatic nerve) . | .33 " | 15 | Tashiro |
| (*Rana temporaria* [2]) (isolated muscle) . . . . . | .18 " | 21 | Fletcher |
| Dog . . . . . | 1.325 " | ... | Regnaut and Reiset |
| Man at rest . . . . . | .41 " | ... | Pettenkoffer and Voit |
| " " " . . . . . | .61 " | ... | " " " |
| " " " . . . . . | .37 " | ... | Speck |

[1] All the figures are quoted from Schäfer's Text Book of Physiology i, pp. 702, 707 and 708, except that of the isolated muscle which I calculated from Fletcher (*loc. cit.*). Fletcher fails to state the weight of a leg, but gives the value .2 c.c. for one-half hour. Hill believes that if we take each leg 6 g. in average, the value will not be far from the truth.

[2] Fletcher fails to state the species of the frog, but it is inferred from Hill's paper.

[38] See the last columns of Table I and Table II.

**Active Nerves.** — That the nerve increases its $CO_2$ production approximately 2.5 times when stimulated, is in accordance with our conception of the metabolism of other acting organs. Just how much increase of $CO_2$ takes place during functional activity of an organ or organisms depends on conditions as well as on habits of different organs and animals. Pettenkofer and Voit [39] report that a man (weighing 70 kgs.) gives off when working 0.76 grams per kg. per hour, while resting only .56 gram. Barcroft [40] found that the submaxillary gland when stimulated by the chorda tympani gives off 3–7 times more $CO_2$ than the resting gland. In the case of contracting muscle, the results are very contradictory. Hermann [41] found that the contracting muscle gave off 9.3 per cent of $CO_2$ (by volume) while the resting one, only 1.4 per cent. Tissot [42] and other workers also found a similar increase of $CO_2$ from contracting muscle. Minot,[43] working with Ludwig, maintains that there is no relation whatever between $CO_2$ production and muscle tetanus. L. Hill [44] and Fletcher [45] both confirmed Minot's work by finding no increase of $CO_2$ production from muscular tetanus. According to Fletcher, the increase he found in $CO_2$ production from a contracting muscle in a closed vessel is due to the rigor. Under this condition, he believes, increased formation of lactic acid is responsible for liberating $CO_2$ already produced. In either case, it is understood that functional activity in the muscle is accompanied by an increase of metabolic activity. It is difficult to compare this increase of metabolic activity of the muscle with that of the nerve unless we determine how much and what kind of metabolism takes place in contracting muscle.

**Respiration Quotient of the Nerve Fibre.** — As quoted before Haberlandt found that a resting nerve consumes 41.7 to 83.4 cmm. $O_2$ for 1 gm. for an hour at 19° – 24°. Although he has not determined chemically the production of $CO_2$ he could easily read the respiration quotient by means of the index fluid. Thus he found

[39] PETTENKOFER and VOIT: *loc. cit.*
[40] BARCROFT: Ergebnisse der Physiologie, 1908, vii, p. 735.
[41] HERMANN: Stoffwechsel der Muskeln, Hirschwald, Berlin, 1867.
[42] TISSOT: Archives de physiologie, 1894–5, (5) vii. p. 469.
[43] MINOT: Arbeiten aus der physiologischen Anstalt zu Leipzig, 1868, p. 1.
[44] L. HILL: See Schäfer's Text Book of Physiology, 1898, i, p. 911.
[45] FLETCHER: Journal of physiology, 1898–9, xxiii, p. 68.

that the respiratory quotient of the resting and acting nerve is nearly unity. Since he found that $O_2$ consumption is increased when stimulated, and since the respiration quotient remains constant before and after the stimulation, he concluded that it must give off more $CO_2$ when stimulated. It is very interesting to compare the $O_2$ consumption in this experment with the $CO_2$ production of mine.[46]

Taking his lowest figure, because he worked in 19° – 24° and I in 19° – 20°, 41.7 cmm. of $O_2$ amount to .00007 cc. for 10 milligrams for ten minutes. My figure of $5.5 \times 10^{-7}$ grams for the same units may be translated to .00027 cc. of $CO_2$ (ignoring temperature and pressure correction). Therefore $\frac{CO_2}{O_2} = \frac{00027}{00007} = 3.8$, the respiratory quotient. As I have not determined $O_2$ consumption of the nerve of Rana pipiens, this figure has no particular value, but the fact that the $CO_2$ production is comparatively higher than $O_2$ consumption is a matter of considerable interest.

One of the most important observations made by A. V. Hill [47] is the fact that he could not detect any rise of temperature in a frog's nerve as measured by an apparatus which is sensitive to a change of one-millionth of a degree. From this, according to his calculation, he concludes that not more than one single oxygen molecule in every cube of nerve of dimension of 3.7 $\mu$ can be used up by a single propagated nerve impulse. Therefore, he suggested that an impulse is not of irreversible chemical nature but a purely physical change.

Although, I confess, my ignorance makes it impossible to interpret his valuable results from my observations, I may add that these two apparently irreconcilable facts may throw light on the true nature of nervous metabolism. Dr. Mathews has suggested that metabolism in the nerve may be something of the order of alcoholic fermentation, which is not a direct oxidation, and where heat production cannot be so large as $CO_2$ production, since the energy content of glucose is only a trifle higher than that of the alcohol produced. The comparatively little heat production in the case of working glands is a matter of interest in this connection. At any rate we should not forget the

[46] He used Rana esculenta, which, by the way, gives for the whole animal .082 g. $CO_2$ per kg. per hour at 17° according to Schultz. My frog was Rana pipiens.

[47] HILL: Journal of physiology, 1912, xliii, p. 433.

anatomical as well as the chemical differences between muscle and nerve. In this respect the ratio between $CO_2$ production and $O_2$ consumption from the nerve is suggestive.

The extremely small intake of $O_2$ has another point of interest in relation to the general nature of irritability. It has been repeatedly reported that a nerve can remain excitable several hours in an oxygen-free atmosphere, although there is no doubt its excitability diminishes, yet there is a considerable amount of evidence to show that oxygen is very closely associated with the state of excitability. To harmonize these two facts, the oxygen-storage hypothesis has been suggested, by which the exhaustion is attributed to complete consumption of the stored oxygen and that excitability is restored when atmospheric oxygen is readmitted. Without committing ourselves to this hypothesis, I may add that according to Haberlandt's figure, the resting nerve of 10 milligrams will consume only .0042 cc. $O_2$ in ten hours. If we take our figure and assume that one volume of oxygen was necessary to produce one volume of $CO_2$ (this assumption is made without any significance except to give a liberal estimate), the $CO_2$ production would require about .015 cc. of $O_2$ for ten hours. And if we assume again that activity will increase $O_2$ consumption in proportion of $CO_2$ production, then it means that the nerve when stimulated takes up only .03 cc. of $O_2$ during ten hours stimulation. I am not aware, at present, of the existence of any method which will surely remove $O_2$ as completely as this from a large vessel; and this is a very liberal estimate. My experiences in rendering the air free from $CO_2$ encourages me to raise the question, How can one remove every trace of $O_2$ from a nerve fibre? Without having a correct criterion for an oxygen-free medium we cannot at present consider definitely any question of the relation of $O_2$ to irritability.

## Conclusion

In spite of all the negative evidence against the presence of metabolism in the nerve fibre, we have established three important facts: namely, (1) A resting nerve gives off a definite quantity of carbon dioxide; (2) stimulation increases $CO_2$ production; and (3) $CO_2$ production from the resting nerve proportionally decreases as irri-

tability diminishes. These facts prove directly that the nerve continuously undergoes chemical changes, and that nervous excitability is directly connected with a chemical phenomenon. There is still another question left, namely, Is there any direct relation between excitability and tissue respiration? To put this question more directly, we may ask: Does excitability depend on the respiratory process in the protoplasm? To answer these questions we must refer to two facts; namely the direct relat on between the rate of respiratory activity and the decrease of excitability; secondly, the influence of reagents on $CO_2$ production and their effects on the state of excitability.

By the studies of $CO_2$ production by Fletcher [48] lactic acid formation by Fletcher and Hopkins,[49] and heat evolution by A. V. Hill,[50] it has been established that in isolated muscle, respiratory processes decrease when irritability diminishes. In the case of the nerve, as shown in Table 3, $CO_2$ production reaches this minimum when excitability approaches zero. These relations, however, do not show conclusively that the protoplasmic irritability depends on respiratory activity, for it is quite probable that the dying nerve may alter its physical condition as well, which according to the physical school, may consequently alter the state of excitability.

That irritability is independent of the respiratory processes has been hitherto successfully contended in the case of the dry seed. The works of Horace Brown, Thiselton-Dyer [51] and others indicate that the dry seed can be kept alive at the conditions where no ordinary gaseous exchanges are possible. It is argued, therefore, that life is possible without any metabolic activity.[52] While a definite potentiality for irritability may exist without any metabolic activity, yet that the irritability can persist without respiratory activity, or vice versa, is a matter by no means settled. In the case of ordinary air-dry seed, Waller could demonstrate the response of electrical changes when stimulated although the detection of $CO_2$ was impossi-

[48] FLETCHER: *loc. cit.*

[49] FLETCHER and HOPKINS: *loc. cit.*

[50] A. V. HILL: *loc. cit.*

[51] THISELTON-DYER: Proceedings of the Royal Society, 1897, lxii, p. 160; *ibid.*, lxv, p. 361.

[52] I am indebted to Professor Crocker for his kind suggestion as to botanical literature.

sible. This failure, however, as he himself expected, was due to the lack of delicacy of the chemical methods for detecting $CO_2$. I observed, with my apparatus that even a single kernel of a dry seed gives off a definite quantity of $CO_2$ as long as it is alive. In ordinary condition not only a living dry seed gives off more $CO_2$ than the dead one, but also like the nerve, it always gives off more $CO_2$ when stimulated by mechanical injury. In the normal condition, therefore, we may safely conclude, there is always metabolic activity as long as the seed is irritable, and that in the different states of irritability, the respiratory activity is proportionately different. At present, therefore, we have no decided evidence which will prevent us from considering excitability as a function of respiration under ordinary conditions. This relation is more directly studied by the use of anaesthetics.

I have already demonstrated that an etherized nerve gives off considerably less $CO_2$ than the normal. Such an etherized nerve will not give more $CO_2$ when it is crushed. This may be interpreted by some to mean that the etherized nerve may be already dead. This, however, is not the case. This objection, also, I have considered by studying the nerve treated with KCl.

When the nerve is treated with .2 m KCl and then crushed, it does not give an increase of $CO_2$ production. Mathews has shown that while a .2 m. KCl solution renders the nerve unexcitable, yet it will recover its excitability by being replaced into n/8NaCl. These two facts, therefore, support the idea that any agents that suppress excitability of the nerves also decrease the $CO_2$ production and that $CO_2$ production by crushing the nerve must be largely due to stimulation. This hypothesis is strikingly supported by similar observations on the dry seed. Etherized seeds give much less $CO_2$ and cannot be stimulated to give more $CO_2$ by crushing, while under normal conditions, crushing a seed always increases its $CO_2$ production. Quantitative experiments in this direction will be given in another paper.

These facts directly support Mathews' hypothesis that substances which suppress irritability must act on the tissue respiration primarily. If such an hypothesis is correct, we can easily picture what is happening in the nerve fibre. Vernon[53] considers that a tissue contains certain substances which can absorb oxygen from their sur-

[53] VERNON: Journal of physiology, 1909–10, xxxix, p. 182.

roundings to form an organic peroxide, and by the help of a peroxidase can transer this to amino acid and carbohydrate molecules bound up in the tissue, just as $H_2O_2$[54] can oxidize, with the help of an activator, an acid of formula R. $CHNH_2$ COOH to $CO_2$, $NH_3$ and an aldehyde RCHO, and then oxidize this aldehyde to RCOOH and ultimately to $CO_2$ and $H_2O$. Poisons such as HNC, $NaHSO_3$ and NaF, which he found to decrease $CO_2$ production, temporarily paralyzed respiration, he thought, by uniting with aldehyde groups, while formaldehyde, acid and alkali temporarily paralyze $CO_2$ forming power of the tissue by destroying the peroxidase. The organic peroxide, though it can still affect some oxidation, cannot of itself carry it to the final $CO_2$ stage. Recovery of $CO_2$ forming power is due to the regeneration of the peroxidase.

Although I doubt that such a process occurs in nervous respiration, the idea of two similar metabolic phenomena involved in the nervous metabolism is very helpful to understand the behavior of the nerve during continued activity. Most recently Tait discovered that a refractory period has two phases, absolute and relative.[55] When he treated the sciatic nerve of a frog with yohimbine, the relative phase is greatly prolonged, while the absolute one is little affected, a result quite different from other common anaesthetics. Waller[56] has already observed that protoveratrin slows up the positive variation of the nerve, while the negative variation is little influenced. Waller contends that this drug does not alter catabolic change, but retards anabolic activity to a considerable degree. Since pharmocological action on animals of protoveratrin and yohimbine are very similar, Tait concludes that these drugs must attack the nerve in similar manner, and that a refractory period, too, must consist of two phases corresponding to the catabolic and anabolic processes which Waller observed in the case of protoveratrinized nerves. Thus, he considers that his "absolute phase" of the refractory period corresponds to negative variation or catabolic process of the nerve, and the "relative" to the positive return or anabolic. Yohimbine, in other words, retards anabolic processes considerably, thus prolonging the refractory period, or increasing nerve

[54] DAKIN: Journal of biological chemistry, 1908, iv, pp. 63, 77, 81, 227.
[55] TAIT: Journal of physiology, 1912, xl, p. xxxviii.
[56] WALLER: Brain, 1900, xxiii, p. 21.

fatigue easily. These considerations suggest very strongly that the absence of fatigability in the nerve as measured by the ordinary methods, is not a question of absence of metabolism, but merely the speed by which these two processes come to an equilibrium.

Although we have an infinite number of facts still unexplainable, by our present knowledge of nerve physiology, we have established a few new facts around which we may build up some idea concerning this most essential phenomena of living matter, — i.e., irritability. As to the true nature of the nerve impulse, I can only confess my ignorance.

## Summary

1. All nerve fibres give off $CO_2$. The resting, isolated nerve of the spider crab produces $6.7 \times 10^{-7}$ gram per 10 milligrams per ten minutes. The frog's sciatic $5.5 \times 10^{-7}$ grams.

2. When nerves are stimulated they give off more $CO_2$. The nerve of the spider crab claw produces $16. \times 10^{-7}$ gram when stimulated, the frog nerve $14.2 \times 10^{-7}$ grams. The rate of increase of $CO_2$ by stimulation amounts to about 2.5 times.

3. The $CO_2$ output of resting nerve is due to a vital active process.

4. Anaesthetics greatly reduce the carbon dioxide output of nerves and dry seeds.

5. Mechanical, thermal and chemical stimulation also increases the carbon dioxide output of nerves.

6. Single dry living seeds (oat, wheat, etc.) react in most particulars similar to nerves as regards their irritability, relation to anaesthetics, mechanical stimulation and carbon dioxide outputs.

7. The general conclusion is drawn that irritability is directly dependent upon and connected with tissue respiration and is primarily a chemical process. These results strongly support the conception that conduction is of the nature of a propagated chemical change.

To Prof. A. P. Mathews, under whose direction I have carried on these experiments, I express my appreciation and gratitude. For many suggestions, I am under obligation to Dr. F. C. Koch.

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# A NEW METHOD AND APPARATUS FOR THE ESTIMATION OF EXCEEDINGLY MINUTE QUANTITIES OF CARBON DIOXIDE[1]

By SHIRO TASHIRO

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IN connection with the study of the metabolism of the nerve fibre, I undertook, at the suggestion of Prof. A. P. Mathews, to work out a method for the detection of exceedingly minute quantities of carbon dioxide. Following a suggestion made by Dr. H. N. McCoy, a very simple method was devised, which I reported first to the Chicago Section of the American Chemical Society;[2] later in conjunction with Dr. McCoy, its further details were reported to the Analytic Section,[3] of the Eighth International Congress of Applied Chemistry. The principle of the new method is as follows:

1. Exceedingly minute quantities of carbon dioxide can be precipitated as barium carbonate on the surface of a small drop of barium hydroxide solution.

2. When a drop of barium hydroxide is exposed to any sample of gas free from carbon dioxide, it remains perfectly clear, but when more than a quite definite minimum amount of carbon dioxide is introduced, a precipitate of carbonate appears, detectable with a lens.

3. By determining, therefore, the minimum volume of any given sample of a gas necessary to give the first visible formation of the precipitate, its carbon dioxide content can be estimated accurately, since this volume must contain just the known detectable amount of carbon dioxide.

[1] One of these apparati was described at the biochemical section, Eighth International Congress of applied chemistry, September, 1912; see also, Journal of biochemistry, 1913, xiv, p. xli.

[2] May 18, 1912.

[3] Original Communication: Eighth International Congress of applied chemistry, 1912, i, p. 361.

I have constructed two apparati, based on this principle, which are especially adapted for the estimation of the output of carbon dioxide for very small biological specimens. With these apparati, one cannot only detect easily a very small amount of gas, given off by a small dry seed, or a small piece of a frog's sciatic nerve, but can also estimate it with considerable accuracy.

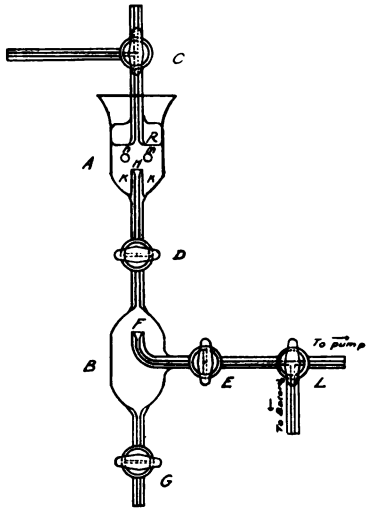



FIGURE 1
One-third the actual size.

The apparatus shown in Fig. 1 consists of two glass bulbs. The upper bulb A, is a respiratory chamber, having a capacity of about 15 c.c., which can be diminished to 9 c.c. by means of mercury. The lower bulb B is an analytic chamber with a volume of 25 c.c., which can be made to 5 c.c. by filling up with mercury. These two bulbs are connected with a capillary stop-cock D. The respiratory chamber is fitted with a tight glass stopper, R, which is connected to a three-way capillary stop-cock C. This glass stopper is so arranged that the chamber can be sealed by putting mercury above the stopper.

The tubes are thick walled capillaries of about 1 mm. internal diameter, excepting upturned tubes inside the bulbs, which should be rather thin walled, especially at F and H, where it is widened to an internal diameter of about 2 mm. It is important that the glass of which these tubes are made should be of a quality not readily attacked by barium hydroxide.

The details of the method of procedure are as follows:

The apparatus is first cleaned and dried.[4] The specimen is

[4] The apparatus is made in such a way that it can be cleaned and dried in ten minutes without being taken apart. For this, the stop-cock D is closed and E and L are opened. The arm at L is connected to the suction pump. Then a little acidulated water is introduced through G. By closing E, and opening D and G, the excess of water is drained off. Then the process is repeated with distilled water, alcohol, and alcohol ether. The last drying is completed by passing a current of air through G while D is closed.

placed on a glass plate [5] and weighed. The glass plate is hung on n and m, which are electrodes fused into the side of the respiratory chamber A. The chamber is now closed with the stopper R and sealed with mercury. Through L, a connection is made with a pump [6] and about 20 c.c. of mercury is introduced through G. Not too much mercury should be used; its surface should not be within 5 mm. of the cup F. Then wash the whole apparatus with carbon dioxide-free air,[7] which is introduced through C, by successive evacuations. After the evacuation and washing out with pure air, which is repeated three or four times, the pressure inside of the bulbs is made equal to the atmospheric pressure by adjusting it at the nitrometer in the usual fashion. Stop-cock E is then closed, and the space between E and L is evacuated so that the barium hydroxide can rush in, a process which is very advantageous to obtain a clear barium hydroxide solution. Then clear barium hydroxide solution is run in through L. By opening E very slowly and carefully, the solution is now introduced into the chamber so that a small drop stands up upon the upturned end of the capillary at F. Then the connection between the two chambers is closed by D. It is imperative that this drop of the solution should be perfectly clear at the start. If no deposit of barium carbonate forms on the surface of the drop within ten minutes,[8] a portion of the sample gas is drawn into B by withdrawing mercury through G and opening the stop-cock D. The volume of mercury withdrawn, which may be readily determined by volume, or more accurately by weight, gives the volume of the sample

[5] The kind of glass plate used in connection with the nerve and small animals like Planaria is shown on p. 120, Fig. 1. (The first paper.)

[6] The pump should be capable of giving a vacuum of at least 25 or 30 mm. of mercury.

[7] Air cannot be freed completely from carbon dioxide by passing it through wash bottles. In my work, carbon dioxide-free air is prepared by shaking air with twenty per cent solution of sodium hydroxide in a tightly-stoppered carboy, fitted with suitable tubes. When this is to be used, it is driven into a nitrometer which is filled with less concentrated alkaline solution (a weak solution is used so that the chamber may not be too dry) by displacing it by running in a solution of sodium hydroxide. After each evacuation, this air is introduced from the nitrometer into the chamber A through stop-cock C.

[8] If no precipitate appears within ten minutes, it is a sure control that the apparatus is free from carbon dioxide.

gas taken from the respiratory chamber, since the pressure in A and B is kept equal to the atmospheric during the transfer.

One now watches the surface of the drop at F with a lens to see whether any formation of barium carbonate occurs within ten minutes. With this apparatus, I have repeatedly introduced accurately known quantities of carbon dioxide of very high dilution into B in the manner just described and as a result have found, with remarkable regularity, that $1.0 \times 10^{-7}$ gram of carbon dioxide is the minimum amount which will cause a formation of barium carbonate within a period of ten minutes. Smaller amounts of carbon dioxide give no visible results; while larger amounts give a deposit more rapidly, and appear in larger quantities. This minimum detectable amount $1.0 \times 10^{-7}$ gram is about the amount which is contained in $\frac{1}{6}$ c.c. of natural air, in which we assume 3.0 parts of carbon dioxide in 10,000 by volume.[9]

In order to determine the concentration of carbon dioxide in the respiratory chamber, one must first find, for the apparatus used, the minimum detectable amount of carbon dioxide. Then one finds, by trial,[10] the minimum volume of gas necessary to give the first visible formation of barium carbonate. This volume must, therefore, contain the known minimum detectable amount of carbon dioxide. From the ratio between this volume and the original volume of the respiratory chamber, out of which this amount is withdrawn, the absolute

[9] LETTS and BLAKE: Proceedings of the Royal Dublin Society, 1899–03, ix, p. 107.

[10] In the case of biological problems, when the specimen gives off carbon dioxide continuously, and sometimes at different rates, varying with the time, it is much simpler not to attempt to determine the minimum volume by a continuous trial with the same sample; but instead to repeat the experiments with a series of samples of known weights for a known time, and determine the minimum volumes which give the precipitates, and the maximum volumes which do not give the precipitates. In this way, it can easily be calculated what is the minimum volume which gives the precipitate for the given weight of the specimen for a given time. Table I on page 114 will illustrate this more clearly.

Another upturned cup H provided in the respiratory chamber A is used in case only the qualitative detection of $CO_2$ is wanted. In such a case, the perfectly clear barium hydroxide solution is introduced, after the necessary cleaning and washing, to the respiratory chamber, forming the usual drop at H instead of F. It should be noted that in case a smaller capacity is necessary for the respiratory chamber, the mercury is introduced by a pipette to the bottom of the chamber at K.

quantity of carbon dioxide, given by the specimen, may be computed.

At the suggestion of Dr. F. C. Koch, another apparatus was constructed, which provides a control drop of the barium hydroxide solution, side by side with the other. The apparatus (Biometer) shown in Fig. 2, although it appears complex, is nothing more than apparatus 1, inclined 90°, but each of its chambers is provided with a barium hydroxide cup d and f. It is made of glass consisting of two respiratory chambers, serving also as analytic chambers, connected by a three-way stop-cock L, the other arm of which is connected to one arm of another three-way stop-cock K. Each of the other two arms of stop-cock K is connected to a nitrometer W and X. The nitro-

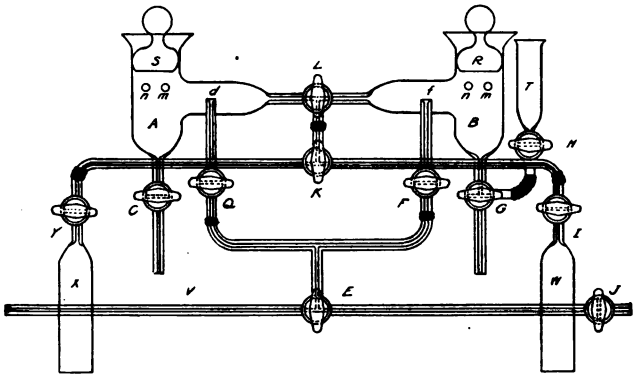


FIGURE 2. Biometer, one-third actual size.

The shaded portions of the apparatus indicate the rubber connection which is first coated by shellac, and then sealed with a special sealing wax. Some parts are also sealed with mercury.

meter on the right, is connected to a carboy with air free of $CO_2$; and the other, on the left, to a similar reservoir with air free of $CO_2$ plus any gas which is desired as a medium for conducting the experiment. Chamber A is drawn to a capillary stop-cock C; chamber B is drawn to the three-way stop-cock G, one arm of which is connected with a mercury burette T, which is used for adjusting the pressure. Both of the chambers have a capacity of 20 to 25 c.c. and are provided with a pair of platinum electrodes n and m, and also with the glass stoppers S and R, which can be sealed as usual with mercury. The pump is connected through J, and the barium

hydroxide solution is introduced through V to d and f, where drops are formed as before.

As stated above, this apparatus can be used for the combined purposes of qualitative detection, quantitative estimation, and comparative determination of the output of $CO_2$ from the various biological specimens. It has a decided advantage over the other in the fact that we have a control drop, side by side, under exactly the same conditions, and that the comparative estimation of $CO_2$ produced by different specimens can be made very easily and accurately. The detailed method of procedure is described under three different headings:

(a) **For the Qualitative Detection of Carbon Dioxide.** — After the apparatus is cleaned and dried,[11] a weighed tissue is placed on the glass plate and hung on n and m of the chamber A, and no tissue in the other chamber. After both chambers are closed with the stoppers S and R and sealed with mercury, they are so filled with mercury that the remaining volumes in both chambers are now exactly the same. The chambers are now evacuated and washed with pure air. When evacuation and washing with pure air is complete, the pressure is made atmospheric, by adjusting with the nitrometer the connection between A and B is then closed with stopcock L. If any $CO_2$ is given off by the tissue, the desposit of carbonate will soon appear on d, while in the control chamber the drop on f remains perfectly clear. In order to avoid any possible error of a technical nature this experiment is repeated by exchanging the chambers, now using chamber B for the respiratory chamber and the other A as a control.

(b) **For Comparative Estimation of $CO_2$ from Two Different Samples.** — By repeated quantitative experiments, it was found that the speed with which the first precipitate appears and the sizes of the deposits on the drops at d and f represent corresponding quantities of carbon dioxide. Thus with remarkably simple means, we can determine simultaneously the comparative outputs of the gas from two different tissues or from the same tissues under different conditions. The method of procedure is best illustrated by the following example. Two pieces of the sciatic nerve are isolated from the same frog and exactly weighed. One piece is laid on one glass plate, and the other

[11] This, too, can be cleaned and dried without being taken apart. See footnote on p. 138.

on the other plate in such a way that one part of the nerve lies across the electrodes of the glass plates as shown in Fig. 1, page 120. In this way, when the plates are hung on the electrodes n and m, any desired nerve can be stimulated with the induction current. These plates are now hung on the electrodes in each chamber, and the usual procedure is followed for the cleaning and the washing of the apparatus to make it $CO_2$ free. After the connection between the two chambers is closed by means of stop-cock L, the nerve in chamber A is stimulated by the current. Then if one can watch over the surfaces of the drops carefully from the start, he finds the first deposit of the carbonate on cup d of chamber A in which the stimulated nerve is placed. Later, the total amount of the precipitates grows much larger in the case of this cup. This increased output of the carbon dioxide from the stimulated nerve, thus observed, can be duplicated by repeating the similar experiment, after exchanging the chambers, as usual. This comparative estimation can be more accurately made by exact quantitative measurement, the method for which the following will illustrate.

(c) **For Quantitative Measurement of Gas.** — The detailed method is exactly analogous to that of apparatus 1. Here we use chamber B as the respiratory chamber and A as the analytic chamber. Barium hydroxide should be introduced into chamber A only at d, and the stop-cock F is always closed except at the time of washing. The pressure should be adjusted by mercury burette T, or by the potash bulb of the nitrometer. In case the mercury burette is used, the remaining volume in the respiratory chamber should be recorded.[12] The introduction of a known amount of gas from the respiratory chamber B to the analytic chamber A is accomplished by withdrawing the mercury from C into a very narrow graduated cylinder, while the stop-cocks L G and H are opened. After a quick adjustment of the mercury burette to equalize the pressure, the stop-cock L is closed and the presence of carbonate is looked for exactly in the same manner as described in connection with the other apparatus, determining the minimum volume that gives the precipitate for the known mass of tissue for a known time.

[12] The bulbs are marked at the point where their capacity became 15 c. c. by introducing mercury. The variation of capacity can easily be read by noting the mercury burette.

In summarizing, I may emphasize the following points:

1. Particular care must be taken to test the air-tightness of the apparatus.

2. Purifying the air must be done with greatest care, as this is essential.

3. The apparatus must be perfectly dry.

4. A weak suction pump cannot be compensated by frequency of washing.

5. As long as the ratio between the c.c. taken from the chamber and the original volume of the chamber is needed, it is most important to have the pressure in A and B equal to the atmospheric. If this is accomplished we can neglect any caution against pressure and temperature variations — a correction which is always necessary for ordinary methods of analysis of exceedingly minute quantities of any gas.

In devising this method and in constructing this apparati, I am under great obligation to Professors McCoy and A. P. Mathews and to Dr. F. C. Koch.

In order to test the accuracy with which an estimate of concentration of carbon dioxide could be made, many determinations were carried out with samples of air which contained accurately known concentrations of carbon dioxide prepared by Dr. F. C. Koch. The experimenter did not learn the concentrations of the samples until after the analysis had been completed. In making up the test samples, pure carbon dioxide, made by heating sodium bicarbonate was diluted with the carbon dioxide free air several times in succession, as illustrated by the following example: 5.5 c.c. of pure carbon dioxide was diluted to 52.0 c.c. over mercury and thoroughly mixed; 5.5 c.c. of the first mixture was diluted to 52.0 c.c.; 1.1 c.c. of the second was diluted to 50.7 c.c.; of this third mixture 5.6 c.c. was received from Dr. Koch. I diluted this a fourth time to 255.6 c.c. to form a mixture to be analyzed. The following observations were made: 0.5 c.c. was introduced into the apparatus and produced no precipitate in ten minutes; 0.5 c.c. more of the same sample, gave no precipitation in another interval of ten minutes; 0.5 c.c. more, a total of 1.5 c.c., was run into the bulb. In six minutes the first evidence of a precipitate appeared on the surface of the drop at d of apparatus 2 and in eight minutes was well developed. Since

the amount of carbon dioxide required to give the precipitate is $1.0 \times 10^{-7}$ grams, this amount is contained in 1.5 c.c. of the sample or 1 c.c. contained $6.7 \times 10^{-8}$ grams of carbon dioxide. The amount of carbon dioxide actually contained in the sample was

$$\frac{5.5 \times 5.5 \times 7.1 \times 5.6}{52 \times 52 \times 50.7 \times 255.6} \text{ c.c.} = 6.2 \times 10^{-8} \text{ grams.}$$

In six such determinations, all made with samples the concentration of which were unknown to the experimenter at the time of the analysis, the results given in the following table were obtained:

| Volume of sample required to give a precipitate | Weight of carbon dioxide in one c.c. | |
|---|---|---|
| | Found | Taken |
| 1.0 c.c. | $1.0 \times 10^{-7}$ g. | $0.92 \times 10^{-7}$ g. |
| .5 c.c. | $2. \times 10^{-7}$ g. | $2.3 \times 10^{-7}$ g. |
| .55 c.c. | $1.82 \times 10^{-7}$ g. | $1.83 \times 10^{-7}$ g. |
| 1.5 c.c. | $.67 \times 10^{-7}$ g. | $0.62 \times 10^{-7}$ g. |
| 2.25 c.c. | $.45 \times 10^{-7}$ g. | $0.45 \times 10^{-7}$ g. |

# ERRATA

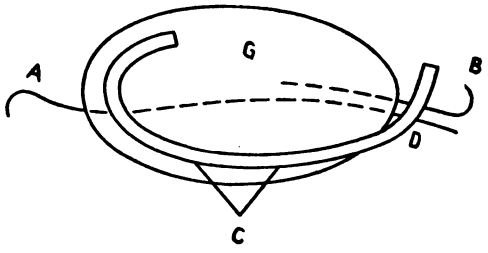
## In June Number of the American Journal of Physiology (Vol. XXXII, No. II)

Substitute "apparatus" for "apparati" in the following places:

Page 110, lines 7, 11, 23.
Page 129, line 1.
Page 137, line 28.
Page 144, line 16.

Substitute "7.1 cc." for "1.1 cc." on page 144, line 29.

In figure 1, page 120, correct as indicated in the following drawing

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Carbon dioxide production from nerve fibres...
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